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RESEARCH NOTE

EXCITATORY AMINO ACIDS HAVE DIFFERENT EFFECTS ON HORIZONTAL CELLS IN EYECUP AND ISOLATED RETINA

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Abstract—Horizontal cells in the mudpuppy eyecup responded to continuous superfusion with L-gluamate, L-aspartate, kainate and quisqualate with a transient depolarization and reduction of the light evoked responses. However, in isolated retina preparations, in which these substances were applied to the photoreceptor side of the retina, the effects were sustained as long as the agonists were present. These results suggest that the transient action of these agonists in eyecup preparations was due to the rapid development of an intraretinal diffusion barrier, and are consistent with the hypothesis that photoreceptors release an excitatory amino acid transmitter.

Retina Horizontal cells Glutamate Aspartate Kainate Quisqualate Mudpuppy Synaptic transmission

INTRODUCTION

There is now considerable physiological evidence that L-glutamate, L-aspartate or some closely related substance is the transmitter released by vertebrate cone photoreceptors (Cervetto and MacNichol, 1972; Murakami *et al.*, 1972, 1975; Kaneko and Shimazaki, 1976; Marshall and Werblin, 1978; Wu and Dowling, 1978). Of the three main types of receptor sites for excitatory amino acids (Watkins and Evans, 1981), the photoreceptor transmitter appears to act on horizontal cells at quisqualate (QA) or kainate (KA) receptors rather than *N*-methyl-D-aspartate (NMDA) receptors (Shiells *et al.*, 1981; Lasater and Dowling, 1982; Slaughter and Miller, 1983; Ariel *et al.*, 1984).

Since horizontal cells receive tonic depolarizing synaptic input from photoreceptors in darkness, continuous superfusion with the putative transmitter ought to produce a sustained depolarization and reduction of light-evoked responses in horizontal cells. However, it has recently been reported by three different laboratories that continuous exposure to excitatory amino acids produces only a transient depolarization and suppression of light-evoked responses in horizontal cells in mudpuppy and turtle eyecup (Shimazaki *et al.*, 1984; Lukasiewicz and McReynolds, 1985a; Normann *et al.*, 1985, 1986). Shimazaki *et al.* (1984) suggested that the transient action of the applied aspartate and glutamate was due to rapid development of a diffusion barrier in the inner nuclear layer, while Normann *et al.* (1986) suggested that the transient effect was due to the removal of the agonists by a cellular uptake system in the outer retina. A third possibility is that the receptor sites on the horizontal cells became rapidly desensitized in the presence of the applied agonists; there is evidence that high concentrations of L-glutamate and quisqualate cause rapid desensitization of their receptor sites on isolated goldfish horizontal cells (Ishida and Neyton, 1985). The last alternative has important implications regarding the nature of synaptic transmission between photoreceptors and horizontal cells: if the transient action of applied aspartate and glutamate on horizontal cells was due to receptor desensitization, then the fact that the light-evoked responses of the horizontal cells were not reduced under these conditions would suggest that the photo-

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receptor transmitter does not act at these receptor sites.

The present study provides evidence that the transient action of applied excitatory amino acids on horizontal cells is most likely due to the rapid development of an agonist-induced diffusion barrier rather than to removal of agonists by uptake mechanisms or to receptor desensitization.

METHODS

Intracellular recordings were made from horizontal cells of the mudpuppy (*Necturus maculosus*), using both eyecup and isolated retina preparations. The electrical recording and optical stimulation have been described in detail previously (Lukasiewicz and McReynolds, 1985b). The eyecup was prepared by decapitating the animal, removing the eye, dissecting away the anterior portion with fine scissors and removing as much as possible of the vitreous humor with a wick of filter paper. The eyecup was mounted with the vitreal side up in the bottm of a plexiglass chamber and superfused with a constant flow of physiological Ringer solution (NaCl 115, KCl 2.5, $CaCl_2$ 1.8, glucose 11, HEPES buffer 5.0 mM, pH 7.8) at a rate of about 1 ml/min. Isolated retina preparations were made under dim light, from animals which had been dark adapted for several hours, by placing the eyecup (prepared as above) vitreous side down on a piece of filter paper. After about 1 min the eyecup was lifted up, leaving the isolated retina attached to the filter paper. The filter paper was then mounted in the chamber with the receptor side of the retina facing up; superfusion and recording were the same as with the eyecup preparation. During the recording period the superfusate could be switched to other solutions to which test substances had been added. Recordings were made with 4 M potassium-acetate filled micropipettes having resistances of 300–600 MΩ. The illustrated responses were photographed from penwriter records. Horizontal cells were identified by their large, hyperpolarizing responses, wide receptive fields, and depth in the retina. The light stimulus was white light from a 100 W tungsten halogen lamp, focussed to a 2 mm diameter spot whose unattenuated intensity at the plane of the retina was $4 \cdot 10^{-2}$ μW/cm^2. The intensities of the stimuli are expressed in $\log_{10}$ units of attenuation by neutral density filters. The light responses of the horizontal cells reported in this study were cone-driven, as evidenced by their waveforms (Fain, 1975; Marshall and Werblin, 1978) and the fact that the sensitivity recovered within less than a minute after supersaturating flashes.

RESULTS

Eyecup preparations

Figure 1 shows the typical effects of excitatory amino acids on horizontal cells in the mudpuppy eyecup. The upper trace shows the response of a horizontal cell to 10 mM L-glutamate. The initial response to glutamate was a depolarization of the horizontal cell in darkness and a reduction in the light-evoked response, as has been previously described for brief exposures to glutamate, aspartate, KA and QA in other preparations (Murakami *et al.*, 1972; Cervetto and MacNichol, 1972; Kaneko and Shimazaki, 1976; Marshall and Werblin, 1978; Ishida and Fain, 1981; Shiells *et al.*, 1981; Slaughter and Miller, 1983; Ariel *et al.*, 1984). However, within less than 1 min the cell began to repolarize and the light-evoked responses became larger; after another 2 min both the dark potential and light responses had returned to near their control values despite the continued presence of the agonist. The middle and lower traces in Fig. 1 show that this phenomenon was also observed with the analogues KA and QA. Similar results were seen in 21 cells with 20–50 μM QA, in 36 cells with 20–50 μM KA, in 10 cells with 5–20 mM L-glutamate, and in 4 cells with 5–20 mM L-aspartate. In 4 cells (2 with glutamate and 1 each with KA and QA) the effects of the agonists were not transient; in these cells the agonist-induced depolarization and reduction of the light response remained for the duration of the exposure. The amplitude of the depolarization produced by a given concentration of each agonist varied considerably among cells, being larger in cells which had more negative resting potentials. However, in all cases the light-evoked responses of the horizontal cell were strongly suppressed during the agonist-induced depolarization.

After the membrane potential and light response of the horizontal cell had returned to normal in the continued presence of the agonist, there was no further change when the superfusate was switched back to normal Ringer solution. Subsequent applications of the same agonist or any of the other agonists within the next few minutes produced little or no response. The length of time required for recovery of

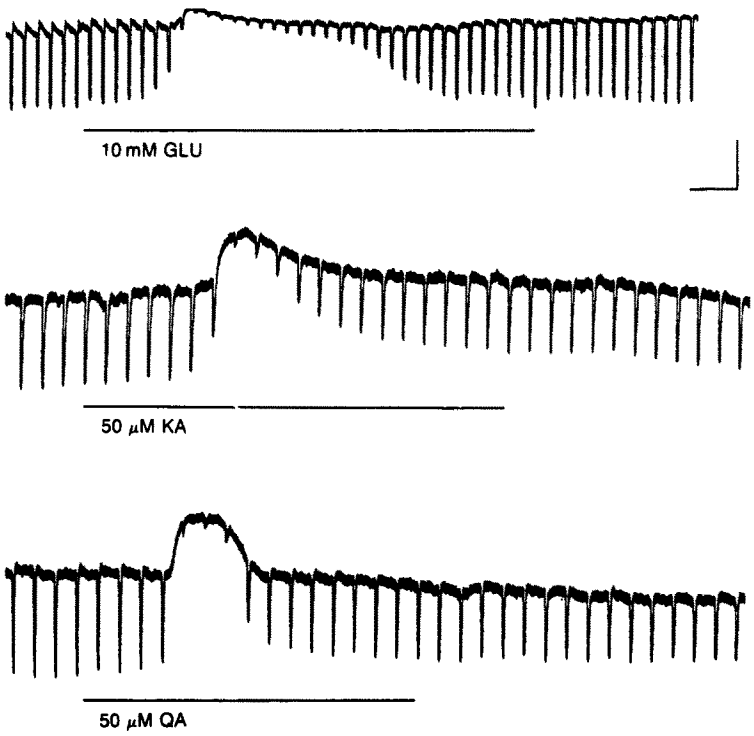

Fig. 1. Responses of horizontal cells in mudpuppy eyecup to L-glutamate, KA and QA. The three traces are from different cells, whose resting potentials in darkness were −27 mV (upper trace), −33 mV (middle trace) and −30 mV (lower trace). In each trace the downward deflections are the responses to light stimuli presented at constant intervals (6 sec intervals in upper trace, 11 sec intervals in middle and lower traces). The horizontal line below each response trace indicates the time during which the superfusate was switched to the indicated test substance. The intensity and duration of the light stimuli were −4.8, 200 msec (upper trace) and −4.8, 500 msec (middle and lower traces). Calibration bars are 12.5 mV, 25 sec for upper trace, and 10 mV, 20 sec for middle and lower traces.

responsiveness to the agonists was approximately 3–5 minutes after the end of the first exposure.

Since Normann *et al.* (1986) suggested that the transient action of applied glutamate and aspartate was due to the removal of these substances by cellular uptake mechanisms in the distal retina, attempts were made to block the uptake with D-aspartate, as described by Ishida and Fain (1981). However, in this preparation 3–10 mM D-aspartate had the same effect as the other agonists described above: it caused a transient depolarization and reduction of light responses in horizontal cells, and in its presence the effects of the other agonists were suppressed.

Continuous exposure to KA, QA, L-aspartate or L-glutamate also caused a transient depolarization of horizontal cells when transmitter release was blocked by the addition of 4 mM Co^{2+} to the bathing medium ($n = 8$), indicating that the return of the membrane potential toward the control level was not the result of synaptic input from another cell type.

Isolated retina preparations

In order to distinguish between desensitization of receptor sites and an intraretinal diffusion barrier as possible causes of the transience of the action of exogenous excitatory amino acids, experiments were also performed using isolated retina preparations. In these experiments the retina was mounted with the receptor side facing up, so that the agonists were applied to the receptor surface instead of to the vitreal surface. This procedure should allow the applied agonists free access to the outer plexiform layer in spite of any diffusion barrier in the inner nuclear layer, but should not prevent desensitization of receptor sites. Figure 2 shows typical responses of horizontal cells in the isolated retina to L-glutamate, KA and QA. All three substances caused a maintained depolarization and suppression of the light-evoked responses; the response was maintained for as long as the agonist was present (the longest exposure tested was 6 min). Similar results were obtained in other isolated retina preparations

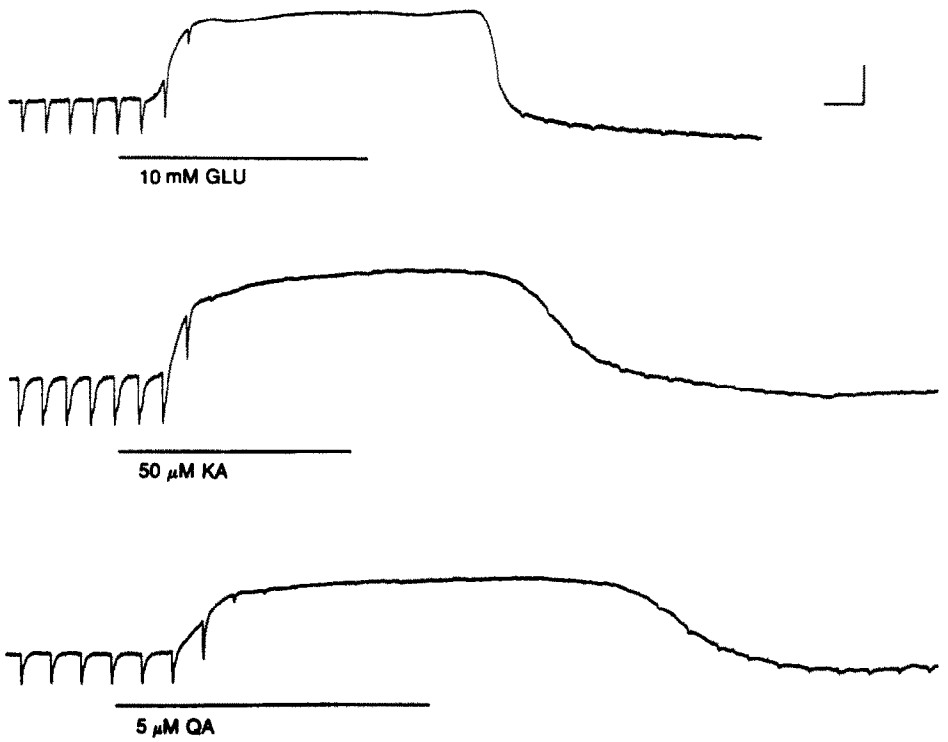

Fig. 2. The effect of L-glutamate, KA and QA on horizontal cells in the isolated retina. The three traces are from different cells, whose resting potentials in darkness were −36 mV (upper trace), −40 mV (middle trace) and −36 mV (lower trace). The intensity and duration of the light stimuli were −5.1, 200 msec (upper trace), −4.8, 200 msec (middle trace) and −5.4, 500 msec (lower trace). Calibration bars are 20 mV, 20 sec for all traces. Other details as in Fig. 1.

with 5–50 μM KA ($n = 22$), 5–50 μM QA ($n = 11$), 1–20 mM L-glutamate ($n = 15$) and 1–20 mM L-aspartate ($n = 8$). In all of these experiments, after removal of the agonist the cell repolarized rapidly to a value that was 5–15 mV more negative than it was prior to agonist application, and then slowly depolarized back to the original resting potential over the next 5–10 min. Although not shown in the figures, the light-evoked responses also recovered fully, roughly in parallel with the recovery of the dark potential from the after hyperpolarization.

DISCUSSION

The finding that the excitatory amino acids produced maintained depolarization and suppression of the light responses in horizontal cells when applied to the photoreceptor rather than the vitreal side of the retina indicates that the transient effect of these substances in the eyecup preparation was not due to desensitization of the receptor sites on horizontal cells. No desensitization was seen with any of the concentrations tested, which were up to 20 mM for L-glutamate and L-aspartate, and up to 50 μM for QA and KA. Although isolated goldfish horizontal cells showed desensitization to L-glutamate at concentrations higher than 100 μM and to quisqualate at concentrations higher than 10 μM (Ishida and Neyton, 1985), no desensitization was observed at lower concentrations (Ishida and Neyton, 1985; Ishida *et al.*, 1984) or in isolated horizontal cells from skate (Lasater *et al.*, 1984).

It is also unlikely that the transient action of the applied agonists in the eyecup preparation was due to their removal by cellular uptake mechanisms in the outer retina, since kainate is not thought to be transported in this way (Lodge *et al.*, 1980). Furthermore, the actions of the agonists were not transient in isolated retina preparations, in which the uptake mechanisms were presumably still operating.

All of the agonists were effective at lower concentrations in the isolated retina than in the eyecup; this difference was also observed for glutamate and aspartate in the tiger salamander retina (Marshall and Werblin, 1978), and is

probably due to some diffusion impediment or uptake mechanism normally present between the vitreal surface and the outer plexiform layer.

Thus, the most likely explanation for the transient action of exogenous excitatory amino acid agonists in the eyecup preparation is the development of an agonist-induced intraretinal diffusion barrier. The sustained agonist effect observed in a few eyecup preparations may have been due to some condition, such as a path along the electrode track, or detachment of the retina at the edge of the eyecup, which allowed the superfusate to reach the photoreceptor side of the retina by an alternate route. Normann *et al.* (1986) noted that the actions of L-aspartate and L-glutamate on horizontal cells in turtle eyecup preparations were maintained rather than transient after the eye was kept at 4°C for 20 h; it is possible that such treatment prevented the rapid development of a diffusion barrier or caused anatomical changes which allowed the agonists access to the receptor sites by alternate paths.

The idea that an aspartate-induced barrier prevented further diffusion of the agonist from the vitreal surface of the retina to the horizontal cells was originally proposed by Shimazaki *et al.* (1984), who observed a large increase in transretinal resistance at approximately the same time as the loss of responsiveness of horizontal cells to aspartate. They suggested that this could be due to swelling of cells in the inner nuclear layer, since similar concentrations of glutamate caused swelling of Müller cells in chicken retina (Van Harreveld, 1982), and also caused spreading depression, which is believed to be associated with cell swelling, in frog retina (Mori *et al.*, 1976). Thus, although in eyecup preparations the actions of exogenous excitatory amino acids on distal retinal neurons may be distorted by the development of an intraretinal diffusion barrier, the results obtained in isolated retina preparations are consistent with the hypothesis that mudpuppy photoreceptors release an excitatory amino acid.

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